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MICROHEMATOCRIT AS A TOOL IN FISHERY RESEARCH AND MANAGEMENT

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MICROHEMATOCRIT AS A TOOL IN FISHERY RESEARCH AND MANAGEMENT

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ABSTRACT

The micro method of hematocrit is rapidly replacing red cell counts in clinical hematology. Observations were made on the value of this method in routine hematological examination of trouts. Under the conditions of data collection, the normal hematocrit values for brook trout were 45 to 50, for brown trout 39 to 44, and for rainbow trout 45 to 53. There was a close correlation between the hematocrits, red cell counts and hemoglobin. The commercial heparinized capillaries, while excellent for human blood, tend to give somewhat higher readings (7 to 18 percent) with trout, due to incomplete prevention of blood coagulation. The procedure as applied to trout is described in detail.

MICROHEMATOCRIT AS A TOOL IN FISHERY RESEARCH AND MANAGEMENT

INTRODUCTION

Hematology is the study of blood or the sum of knowledge about blood. Much of this information consists of measurements of value of the components of blood under normal and abnormal conditions. Most readers have had red and white cell counts or hemoglobin determinations made during routine medical examinations. Less familiar, perhaps, but even more important is the hematocrit determination.

The value and significance of hematological examination, and that of hematocrit in particular, may best be conveyed to readers not familiar with these procedures, by quotations from authoritative sources:

"Since a change or lack of change in the 'blood picture' is a fundamental characteristic of practically every physiologic or pathologic state, hematologic findings are among the most valuable and most generally useful of all laboratory diagnostic aids..... The field of clinical hematology is well within the reach of every practitioner. The laboratory methods are not really demanding, and diagnostic interpretations are generally not difficult" (Wells, 1956).

"Determination of the hematocrit reading, the erythrocyte count and the hemoglobin concentration are all used in evaluating the erythrocyte content of blood. The hematocrit determination is the most accurate of these methods inasmuch as it is not subject to the rather large errors inherent in pipetting and diluting blood according to the other methods" (Strumia et al., 1954).

"The hematocrit reading, or the percentage of packed cells in the peripheral blood is one of the most important of all clinical constants. Because of its simplicity and high degrees of reproducibility, this procedure is most useful as a routine for detection of anemia, (Wells, 1956).

Some aspects of fish hematology have been reported. Hematocrit determinations have been given in some of the more recent works which are here briefly reviewed. The most complete comparative study on the blood of vertebrates was made by Wintrobe (1934) who introduced hematocrit to hematology. He found that hematocrit, hemoglobin, and mean corpuscular hemoglobin concentration were uniform among all vertebrates, but that the number of red cells was the most variable. The hematocrit values in fish range from about 5 to about 60. This unusually great amplitude probably results from the fact that many of the examined fish were kept under abnormal conditions. The author makes it clear, therefore, that his figures should not be considered as representative for the different species of fish he examined. Wintrobe's data show that among warm blooded vertebrates the hematocrit values were mostly between 35 and 50.

Studies on marine fishes reported by Kish (1949) show similar hematocrit values to those found by Wintrobe, but with a narrower amplitude (20-51). Hematocrit values for freshly caught carp (Cyprinus carpio) and northern pike (Esox lucius) are of the same magnitude (Vars 1934). Similar hematocrit values for carp and eastern brook trout (Salvelinus fontinalis) were reported by Field et al. (1943) and for sea-lamprey (Petromyzon marinus) by Thorson (1959). Repeated hematocrit determinations for individual fish during a period of several months show that these values vary considerably. Whenever a fish supposedly became diseased or lost appetite due to undetermined causes, the hematocrit values became much lower (Young, 1949). So far as I could establish, Benditt, Morrison and Irwing (1941) were the first to use capillaries for hematocrit determination in fishes. They found for Atlantic salmon (Salmo salar salar) in brackish water the mean hematocrit value was 39 and in fresh water the value was reduced to 25. Watson et al. (1956) recorded 47 as the normal mean microhematocrit value in fingerling sockeye salmon (Oncorhyncus nerka). In the same lot of salmon apparently infected with a specific pathogenic virus the hematocrit values were significantly lower. Hematocrit values are not included in the recent manual "The Physiology of Fishes" (Brown, 1957).

Hematological examinations of fish are usually made either in the course of research or

used as a tool for quality control in fish culture or management. Selection of methods of hematological examination for research purposes depends on the nature of the investigation and must be left to the individual worker. In order to be used generally as quality control tools, the hematological methods selected must be simple, rapid and as free as possible from procedural errors. Hematological examination may be used to detect some types of malnutrition (Tunison et al, 1939), chronic diseases, or disturbances caused by unfavorable environment or pollution (McCay, 1929). Red cell counts and estimation of hemoglobin have been the procedures used most frequently in the past. Hematocrits were used rarely, because the early hematocrit methods required large quantities of blood which seldom were obtainable from fish of the size raised in hatcheries. A detailed review of reference material pertaining to hematology of fishes can be found in papers by Yokoyama (1947) and Katz (1949).

Critique of the significance and of the limitations of red cell counts, hemoglobin and hematocrit determination can be found in recently published manuals of hematology or clinical pathology, as for example the well known books by Wintrobe (1958)and Wells (1956).

The purpose of this investigation was to determine the practicability of large-scale microhematocrit measurements in fish culture and to establish values which may serve as normal for trout until more extensive data are collected.

Determination of normal values for fish is difficult. Changes in concentrations of dissolved oxygen rapidly affect hematological values in fish (Hall et al., 1926; Adrianov, 1936; Phillips, 1947; Dombrowski, 1953, and others). For this reason hematological standards for fishes are likely to have wide amplitudes. Only a very thorough examination of fishes under different conditions may ultimately result in establishing significant standards within normal amplitudes. Our observations are a contribution to this aim.

While a microhematocrit method was described by Guest and Siler as early as 1934, equipment became commercially available only recently (McGovern et al. 1955). Since then the microhematocrit method has gained general acceptance in clinical laboratories. Procedure described by Guest and Siler requires one drop of blood or 20 to 40 µl (microliters or cubic millimeters). In the ultra-microhematocrit method of Strumia et al. (1954) only 5 to 10 µl of blood are used; thus hematocrits can be performed with fish from which even less than one drop of blood is obtained.

METHODS

The technique we used is essentially that recommended by McGovern et al. (1955). Trout used for this work were hatchery stock kept in water of 12° to 14°C. (54° to 58°F.). They were picked at random, several at a time, and kept in a small tank supplied with fresh and freely flowing water of the same temperature until all were examined. Immediately prior to examination each fish was completely anesthetized by exposure for about one minute in a water solution (approx. 1: 2000) of tricaine methanesulfonate (MS 222). After wrapping in paper toweling to blot the surface and to cover the vent in order to prevent contamination of the sample, the caudal peduncle was rapidly cut off with sharp scissors. Blood which welled from the dorsal vessels was collected in heparinized capillaries 75 mm long and one end closed either in flame or with modeling clay. Capillaries were centrifuged in an International Microcapillary Centrifuge Model MB for 5 minutes and hematocrit determined by means of a plastic reader. (The supernatant plasma is used for other tests).

The usefulness of the hematocrit determination depends upon its speed, accuracy and close relationship to red cell counts and hemoglobin concentration. In order to perform all three tests simultaneously, blood was collected in a spot test plate depression containing a trace of dried anticoagulant. The blood was continuously stirred until all needed pipettes and capillaries were filled. Red cell counts were made in the usual manner and hemoglobin was determined with a hemoglobinometer (Spencer #HB meter No. 1000).

Fish selected for these observations were fingerlings and yearlings of eastern brook trout, brown trout (S. trutta) and rainbow trout (S. gairdneri) fed Cortland diet No. 6 mixed with beef liver and spleen.

For the purpose of comparison, a separate series of hematocrits were run simultaneously in

75 and 32 mm heparinized and plain capillaries. In another series plain capillaries were filled with untreated blood which was permitted to coagulate completely. At the same time blood treated with anticoagulants was also introduced into similar untreated capillaries and both were centrifuged in the same manner and at the same time. Hemoglobin values obtained with the hemoglobinometer were checked with a Bausch and Lomb "Spectronic 20" spectrophotometer.

The presentation of quantitative data in tables including mean values, mean deviations, variances or standard deviations and frequency distributions may seem unnecessary to some readers, but may be of value to others who want to compare statistically their own data with those presented here.

RESULTS

Data presented in this report are based on examination of about 300 trout. Red cell counts and estimation of hemoglobin, in addition to microhematocrit, were made with 64 of the trout examined. In table 1 data are presented on microhematocrits. In most cases to or three hematocrits were determined for each trout. This was done to determine the reproducibility of the hematocrit method when applied to trout blood. The frequency distribution of these replicated hematocrits shows that in 80 to 90 percent of the cases the difference between the replicates is $\pm$ 1 unit or less. This is a very high degree of reproducibility.

The mean hematocrit values for all three species of trout were between 40 and 50, with an average about 45. The frequency distribution of hematocrit values seemed to become less regular with increasing age. It seems likely that this was caused by gonad development in yearling trout, and/or high incidence of mycosis-like granuloma (Wood et al., 1955) observed in yearling eastern brook trout.

The relationship between the hematocrits, red cell counts and hemoglobin as determined in the same trout are presented in table 2 and figure 1. From this it can be seen that as in other vertebrates, there is a correlation between these values.

Hematocrit readings in apparently normal trout differ only slightly from average hematocrit levels in humans and most other vertebrates. It is believed, therefore, that figures obtained for trout at the Leetown hatchery may be tentatively considered "normal" for trout. How representative these figures are for the same species of trout of the same age but with different environmental conditions remains to be demonstrated.

Comparative data on microhematocrit values from rainbow trout blood pre-treated with anticoagulant with that taken directly into two different brands of heparinized capillaries are presented in table 3. Pre-treated samples gave values 18 percent lower than those taken in heparinized capillary tubes.

When fish blood was collected directly into capillaries treated with anticoagulants and such capillaries were kept in vertical position, there was no visible sedimentation of blood cells. However, if blood was treated with the same anticoagulants before introducing into capillaries, blood cell sedimentation proceeded at about the same rate as with similarly treated human blood. Also when untreated and pretreated blood was kept in capillaries for some time and then blown out on a porcelain plate, the pretreated blood was uniformly liquid while blood collected directly into treated capillaries had a gelatinous consistency. Blood collected into plain capillaries was coagulated and the clot was well separated from the clear serum.

Samples of fresh human blood were run at the same time. Blood collected in treated capillaries did not clot. Cell sedimentation was rapid and hematocrit values, regardless of the type of handling, were identical within the range of experimental error.

These observations show that even in treated capillaries some coagulation of trout blood occurs and hematocrit values are somewhat higher than for pretreated blood.

Data are presented in table 4 on hematocrits run with trout blood in 75 mm and 32 mm capillaries (Strumia, 1954). In all cases determinations were made with blood introduced directly into heparinized capillaries and with blood the coagulation of which was prevented by pretreatment with

Table 1.--Microhematocrit values in three species of trout as obtained in commercial, heparinized, 75 mm capillaries.

	Species of Trout								
	Brook			Brown			Rainbow		
	Group			Group			Group		
	I	II	III	I	II	III	I	II	III
Number examined	32	63	30	32	52	12	28	50	11
Age in months	4	8-9	15	3.5	9.5	15.5	7	8-9	15
Mean, length in centimeters	10.1	15.3	23.6	10.2	17.[illegible]	23.0	14.9	14.2	23.5
Mean deviation	0.4	1.8	2.2	1.9	1.9	1.9	1.0	1.5	1.6
Microhematocrit, mean	45.3	48.3	49.7	43.6	39.1	41.1	47	45.3	53
Mean deviation	2.8	5.3	6.9	2.1	3.1	4.5	4.0	3.4	6.8
Variance	13.3	46.8	98.2	7.9	22.4	32.9	28.1	37.1	75.6
Standard deviation	3.6	6.8	9.8	2.8	4.7	5.7	5.3	6.1	8.7
Frequency distribution:	Percent of total								
up to 34	0	0	7	0	14	8	0	2	9
35-39	6	11	7	9	40	17	7	4	0
40-44	31	19	10	47	40	25	22	30	18
45-49	50	29	10	44	0	33	43	54	0
50-54	12	20	37	0	4	17	18	6	9
55-59	0	13	17	0	2	0	9	2	55
60 or above	0	8	13	0	0	0	0	2	9
Replicate hematocrits run from the same fish difference from the mean; Frequency distribution:	Percent of total								
0	42	66	70	43	41	9	46	60	82
1	45	30	17	44	55	73	41	29	18
2	10	7	10	8	4	9	9	8	0
3 or more	3	0	3	5	0	9	4	0	0

Table 2.--Red cell counts, hemoglobin determination and microhematocrit values in three species of trout.

	Species of trout			
	Brook		Brown	Rainbow
	Group I	Group II		
Number examined	11	30	12	11
Age, month	9	15	15.5	15
Mean, length in cm.	14.2	23.6	23.0	23.5
Mean deviation	1.2	2.2	1.9	1.6
Red cells, mean	1,262,000	1,260,000	1,181,000	1,372,000
Mean deviation	108,000	163,000	101,000	145,000
Hemoglobin (grams per 100 ml.), mean	10.1	9.8	8.1	8.7
Mean deviation	0.9	1.3	0.6	1.2
Microhematocrit	46.4	49.7	44.1	52.8
Mean deviation	6.4	6.9	4.5	6.8

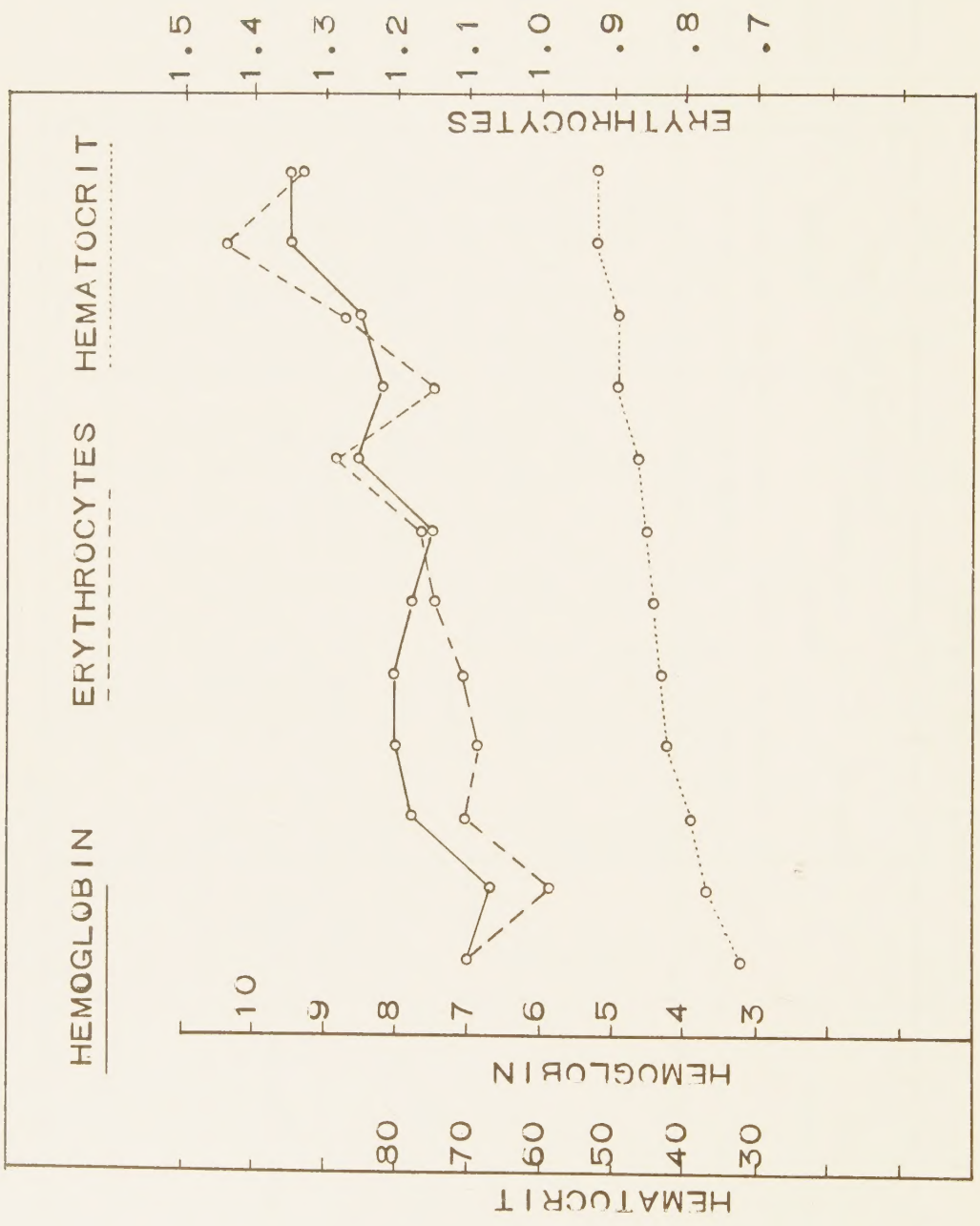

Figure 1:--Correlation of numbers of erythrocytes and hemoglobin levels with hematocrits in yearling brown trout. Hematocrit is expressed as percentage of packed red cells in blood, hemoglobin in grams per 100 ml of blood and number of erythrocytes is expressed in millions. Samples are arranged in order of ascending hematocrit values.

Table 3.--Comparison of microhematocrit values in fingerling rainbow trout obtained with capillaries internally coated with anticoagulants with values obtained with blood pre-treated with anticoagulants before introduction into plain capillaries.

	Heparinized capillaries			Treated with	Blood pre-treated with anticoagulant			
	Aloe		A. Thomas	T.A. 1/	Anticlot 2/		T.A. 1/	
	Run 1	Run 2	Run 2	Run 2	Run 1	Run 2	Run 2	
	44	38	37	37	33	36	34	Mean decrease in pretreated
	48	43	41	41	36	32	35	
	47	45	42	43	34	34	40	
	50	43	43	44	42	35	38	
	43	42	45	45	33	38	35	
	47	46	43	42	36	31	37	
	43	--	45	42	32	34	35	
	40	--	--	--	31	--	--	
Human Blood	44	43	43	43	--	44	42	
Mean	45	43	42	42	35	34	36	18.5%
Mean deviation	2.7	1.8	1.7	1.7	2.5	1.7	1.7	
Variance	9.5	6.2	5.0	5.4	8.8	4.8	4.0	
Standard deviation	3.0	2.5	2.2	2.3	3.0	2.2	2.0	

Trout size in Run No. 1 = Mean 14.3 cm.; mean deviation 1.04 cm.
Trout size in Run No. 2 = Mean 15.5 cm.; mean deviation 0.50 cm.

1/ Ten percent water solution of Dipotassium ethylenediamine tetreacetate.

2/ A heparin anticoagulant. Clinton Laboratories, Los Angles, California.

Table 4.--Comparison of hematocrits run in capillaries coated with heparin and with blood pretreated with heparin before introduction into plain capillaries.

Species and age of trout	Types of data	Blood collected into heparinized capillaries		Blood pretreated with "Anticlot"	
		75 mm	32 mm	75 mm	32 mm
Ten fingerling brook trout, eight months old	Mean	42.0	41.0	39.0 (-7.1%) 1/	38.0 (-7.3%)
	Mean deviation	4.1	3.6	2.2	2.9
	Variance	22.1	17.4	10.0	17.0
	Standard deviation	4.7	4.1	3.1	4.1
	Mean deviation between replicates from the same trout	0.6	1.1	0.6	0.7
Ten fingerling brown trout, eight months old	Mean	35.0	33.0	32.0 (-8.5%)	30.0 (-9.1%)
	Mean deviation	1.7	1.6	1.8	2.1
	Variance	4.7	3.6	5.8	9.1
	Standard deviation	2.2	2.0	2.4	3.0
	Mean deviation between replicates from the same trout	0.7	0.9	0.7	0.9
Five fingerling rainbow trout, eightmonths old	Mean	38	35	33 (-13.1%)	31 (-11.4%)
	Mean deviation	3.0	3.8	3.6	3.0
	Variance	12.0	15.4	16.0	9.4
	Standard deviation	3.4	3.8	4.0	3.0
	Mean deviation between replicates from the same trout	0.6	1.0	0.4	0.7

1/ All figures in parentheses show the percentage of the decrease in hematocrit values when blood was pretreated with heparin before introducing into plain capillaries.

heparin. The data in table 4 show as in table 3 that pretreated blood gave lower hematocrits. It is interesting to note that values were somewhat lower in 32 mm capillaries.

In another series blood from 8 trout of each species was pretreated with heparin in test plates and at the same time blood from the same trout was introduced directly to plain capillaries. After the untreated blood had coagulated in plain capillaries, all capillaries were centrifuged simultaneously and the columns of packed red cells and the columns of packed clot were measured. The results are presented in table 5. They should be compared with those presented in tables 3 and 4. It is evident from this comparison that the commercially available heparinized 75 mm x 1.1 - 1.2 capillaries are not entirely satisfactory for exact determination of hematocrits in trout, giving only slightly better results than plain capillaries. Comparison of these microhematocrits with data shown in table 3, shows that the commercial heparinized capillaries are entirely satisfactory with human blood for which they are prepared.

Hematocrit values with trout blood obtained in commercial heparinized capillaries were found to be 7 to 18 percent higher than readings obtained with pretreated blood in plain capillaries. Therefore until this difficulty is removed, the hematocrit values obtained with the 75 mm 1.1 to 1.2 commercial heparinized capillaries should be corrected accordingly.

Results from determination of hemoglobin in trout blood and oxyhemoglobin are presented in table 6. Data may be of value to persons who are using these two methods for fish blood examination.

DISCUSSION

It has been found with trout, as with humans, that the hematocrit is a simple, accurate and rapid method for detection of ordinary anemia. One must keep in mind, however, that there are many types of anemia (Wells, 1956; Wintrobe, 1958) and it is likely that in an examination of fish blood, determination of hematocrit cannot always be used as the only hematological method. Microhematocrit is as accurate as macrohematocrit (McGovern et al., 1955) it requires only one or two drops of blood and the ultra micro method of Strumia et al. (1954) can be run with even less blood. It is evident from the excellent comparative hematological examination reported by Wintrobe (1934) that hematocrit is a relatively uniform value in all vertebrates and that it correlates very well with red cell counts, hemoglobin and other hematological values. Wintrobe found that differences in hematocrits were very small in comparison to great differences in number and size of red cells. Also the differences in mean corpuscular hemoglobin corresponded inversely to differences in number of red cells. Therefore the amount of hemoglobin per unit volume of blood varied slightly. The mean corpuscular hemoglobin concentration was the most uniform constant in all vertebrates examined. There is a very close relationship between the amount of hemoglobin and hematocrit per unit of blood volume (Wintrobe, 1934). There is little doubt that the microhematocrit is a useful procedure in routine and special examination of fish blood.

In order to use hematocrit for evaluation of condition of fish in hatchery production, management, or research it is necessary to know the "normal" hematocrit values. Most of the humans and land vertebrates live in an atmosphere of uniform composition. The gill breathing animals may be exposed in nature to an extreme variety of environmental conditions, especially availability of oxygen and concentration of carbon dioxide. Therefore one must expect that under different environmental conditions there will be considerable hematological variation in otherwise "normal" animals. While it should be possible to establish the "normal" hematocrit values for different species of fishes, the amplitude of normal values will have to be wider than that for terrestrial and particularly for warm blooded animals.

Amount of dissolved oxygen (Hall et al., 1926; Phillips, 1947; Dombrowski, 1953); season of the year (Ivlev, 1957; Yokoyama et al., 1947), physiological activity (Dubravko, 1956), temperature (Scholander, 1957), pollution (McCay, 1929) and perhaps other factors have a pronounced influence on the morphological and chemical composition of fish blood.

Because even in "normal" fish the composition of blood may show considerable variation, it

Table 5:--Comparison of microhematocrit values obtained with trout blood pretreated with heparin and with untreated blood which clotted in plain capillaries. Both types of blood were centrifuged in identical conditions.

	Species of trout					
	Brook 9 months old		Brown 9 months old		Rainbow 9 months old	
	Heparinized	Not treated	Heparinized	Not treated[1/]	Heparinized	Not treated
Hematocrit, or blood clot, mean	32	38	39	47	45	51
Mean deviation	4.9	6.4	2.0	3.1	5.4	5.2
Percent difference between treated and untreated blood	-15.8		-17.0		-11.7	

1/ The clot was so compact that it would not break away from the wall of the capillaries and separate when centrifuged in the microhematocrit centrifuge unless separated from the walls of the capillaries by means of a fine wire.

Table 6:--Determination of hemoglobin in 10-month old trout by means of two methods expressed as grams of hemoglobin per 100 ml. of blood.

Method of Determination	Species of trout (Ten trout of each species)		
	Brook	Brown	Rainbow
Oxyhemoglobin, mean (B and L Spectronic 20)	7.6	5.7	8.0
Mean deviation	1.5	0.5	1.0
Hemoglobin, mean (Spenser Hb meter No. 1000)	7.8	6.4	8.6
Mean deviation	1.3	0.6	1.0
Difference between the two methods, percent	2.5	11.0	7.0

is necessary to determine the amplitude of such "normal" variation before examination of blood can be used for detection of "abnormal" or pathological conditions. Environmental conditions at different hatcheries must have a significant effect on the composition of fish blood. Therefore for effective use of hematological examination of fish for management purposes two sets of "normal" standards must be established. One should be the general or national standard with a wide range of values, another should be for individual hatcheries.

While microhematocrit cannot replace complete hematological examination for research purposes, it is the only hematological method which is simple, accurate, relatively free from observational and procedural errors, and fast enough to be practical for a general use.[1/] It is suggested that it be used as widely as possible and that the findings be made easily accessible so that in time general and local standards can be established.

Observations here presented show that the commercial heparinized capillaries which we have used gave results 7 to 18 percent higher than when blood was treated with anticoagulants before introduction into capillaries. Such difference has not been noticed with human blood (table 3). This probably is due to much faster coagulation of fish blood and the role played by erythrocytes in blood clotting in fishes, as evident from the recent research and review of this subject by Wolf (1959). Additional work is needed and a study is in progress to develop a treatment for microhematocrit capillaries which will completely prevent clotting and gelation of erythrocytes and in this way obtain more accurate hematocrit values with fish blood.

After a microhematocrit is determined there is about 20 µl of plasma left in each capillary. This may be used for the ultra-micro chemical tests described by Natelson, 1951.

DESCRIPTION OF TECHNIQUE

Performance of the hematocrit test is very simple and rapid. Detailed information given here about the technique and precautions is based on the recommendations made by Mc Govern et al. (1955) and on our personal experience.

1. If the hematocrit is run as a routine periodic test of fish quality in the hatchery, it is extremely important that fish selected for examination are a representative sample from the lot or a population. Sampling techniques are described by Hewitt and Burrows (1948).

2. If the hematocrit is run for diagnostic purposes the sampling technique must be entirely different from that used in evaluation of a lot or a population. For diagnostic purposes fish should not be sampled at random but only individuals in stress or showing abnormalities in appearance or behaviour should be selected. It is also desirable to examine for comparison several fish which appear normal.

3. It has been shown by several authors that the blood picture in fish may undergo profound and rapid changes if fish are exposed to partial asphyxia. Therefore it is important to take the blood sample immediately after fish are collected. If this is not possible, fish should be kept under conditions which are as similar as possible to those existing in the body of water in which they were raised.

4. Fish to be examined should be anesthetized just before taking blood. The anesthetic should be a rapidly acting one, so that no changes in blood will occur while fish are being anesthetized. We are using tricaine methanesulfonate (M.S. 222) in concentrations from 1:2000 to 1:5000 which usually anesthetizes trout in less than one minute.

1/ The microhematocrit method was used on a large scale by fish hatchery biologists of the Bureau of Sport Fisheries and Wildlife in the central and eastern United States during 1959. This method permitted detection of numerous cases of anemia in rainbow trout due either to malnutrition, or an infectious disease, or a combination of both. Remedial nutritional measures permitted the control of this anemia and prevention of heavy losses to some degree. As result of our recommendations many Federal and State hatcheries are using the microhematocrit method in year around quality control of fish.

5. There are many methods of taking blood samples. If fish are very small, it is best to sacrifice them and take blood from the dorsal blood vessels after cutting off the caudal peduncle. In larger fish blood can be taken easily by cardiac puncture or from the aorta in the roof of the mouth (Schiffman 1959). Another way is to cut a gill arch or puncture dorsal blood vessels in the caudal peduncle by a spring-activated blood lancet. Most of the fish survive such operations well. It is very important to have the cut surfaces free from water, the presence of which may dilute the blood sample and cause hemolysis. If blood is taken from the caudal peduncle the vent must be covered so that no excrement contaminates the blood sample. If blood is taken with a syringe, the needle and the syringe must be treated with an effective anticoagulant, but must be free from any liquid which may dilute the blood sample, particularly if the sample is very small. If blood is collected by means of a syringe, a sufficient number of treated and dry syringes with needles should be on hand for the number of fish to be examined at one time.

6. If blood is collected directly to hematocrit capillaries, there are two sizes of commercial capillaries treated with anticoagulant available. The most frequently used capillaries are 75 mm long with outside diameter of 1.2 to 1.4 mm. The other type for the Strumia technique is 32 mm long and 0.8 mm in diamter. When fish are large enough the 75 mm capillaries are preferable. The 75 mm tubes require about 0.03 to 0.04 cub ml of blood and the 32 mm capillary about 0.01 cubic ml (or about 10 µl). If blood is taken from the incision the capillary is filled by holding it horizontally and touching the drop of emerging blood. If blood is collected by means of a syringe it can be collected from the tip of the needle or the tip of the syringe after the needle is removed. If blood is collected in any vessel treated with anticoagulant, it should be well stirred to keep it homogeneous before filling the capillaries.

Capillaries of 75 mm are available without anticoagulant. These should be used when blood is treated with anticoagulant in the syringe or in any other way prior to filling the capillaries. They may also be used if serum is required instead of plasma. The hematocrit reading has no value whatsoever if blood is permitted to coagulate during handling.

7. As soon as capillaries are filled with blood, one end should be well closed. When several capillaries are prepared they may be kept in horizontal position until the last one is filled. Capillaries may be closed either in flame or with modeling clay; other methods of closing are not as good. When closing in flame, capillaries should not be filled more than 2/3 or 3/4 of their length. Only the end which was not in contact with blood and which is not soiled with blood can be flame closed. The flame used should be hot as possible. If no microburner is available, a 20 or 22 gage hypodermic needle is an effective substitute; a butane torch with a fine nozzle is also satisfactory. It should take only a few seconds to close the capillary. The part of the capillary containing blood should not be heated because the blood will break down. To prevent blood from scorching, the capillary should be held horizontally in the fingers in the place where the blood ends. If heat penetrates too far, or the flame is too large, or the capillary contains too much blood, the capillary will be too hot to hold. Until one becomes experienced it is advisable to examine the fused end with a hand magnifier for pinholes.

If modeling clay is used it should not be red but rather a color contrasting with blood. A quarter-size disc, about 1/8 to 1/4-inch thick should be prepared. It should be held in one hand; with the other hand one should insert the capillary, held horizontally, into the disc while rotating it slowly. We have found that the only reliable way of closing the 32 mm capillaries is a very hot pinpoint flame.

As soon as capillaries are sealed they should be inserted vertically, seal down, into a rack. Wooden racks with numbers are commercially available. In this position capillaries with blood can be kept for several hours at room temperature (Guest and Siler, 1934). Whenever work is performed outdoors the capillaries should be kept in shade and should not be allowed to freeze even for the shortest time.

8. The most important step is centrifuging. Any clinical centrifuge may be used, provided that the tubes are protected from breakage during

centrifugation. Centrifuging for 25 to 30 minutes at 3000 rpm is entirely satisfactory. It is recommended, however, that whenever possible a special microhematocrit centrifuge with a head especially designed for capillaries be used. Such centrifuges have a speed of about 12,000 rpm., hold up to 24 capillaries, and are run for 4.5 to 5 minutes.

9. Unless capillaries are properly sealed blood may be lost. To guard against loss, the total length of blood column in each capillary should be measured with an accuracy of 1 or even 0.5 mm, and recorded before centrifuging; after centrifugation it should be re-measured. If the column of cells plus plasma is 1 mm shorter than before centrifuging, some blood has been lost and the capillary should be discarded.

10. As soon as the centrifuge is stopped, capillaries should be removed, placed in vertical position and read. If no loss of blood has occurred, the length of the column of packed red cells and the total length of the column made of blood cells and plasma should be measured as accurately as possible. The very thin, grayish-white layer of leucocytes on the top of the erythrocytes should not be measured if it is less than 1 mm thick. If greater than that it may indicate pathologic conditions and it should be measured and recorded.

There are many types of commercially available hematocrit readers. Entirely satisfactory results can be obtained with inexpensive ruled plastic readers or a millimeter scale.

SUMMARY

The hematocrit value is the percentage of packed red blood cells in an examined sample of whole blood treated with an effective anticoagulant. Hematocrit has a wide application in clinical hematology of humans and animals. Microhematocrits performed with three species of trout at the Leetown hatchery gave the following mean readings: brook trout 45 to 50; brown trout 39 to 44; rainbow trout 45 to 53. The correlation between the hematocrit, red cell counts and hemoglobin is as good in trout as in humans and other vertebrates. Hematocrit readings obtained in heparinized capillaries are 7 to 18 percent higher if compared with hematocrits obtained with blood treated with anticoagulants before filling capillaries. A detailed description of the microhematocrit procedure as applied to trout blood is included.

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